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THE TOXICITY OF 2,4-D AND PICLORAM HERBICIDES TO FISH

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Technical Paper

THE TOXICITY OF 2,4-D AND PICLORAM HERBICIDES TO FISH

TO: J. F. McLaughlin, Director
Joint Highway Research Project

October 28, 1970

File: 9-5-3

FROM: H. L. Michael, Associate Director
Joint Highway Research Project

Project: C-36-48C

The attached Technical Paper "The Toxicity of 2,4-D and Picloram Herbicides to Fish" has been prepared in part from research conducted on the HPR-1 (8) project "Research in Roadside Development", Part III, Chemical Weed Control. The authors are M. Sargent, D. Blazek, J. H. Elder, C. A. Lembi and D. J. Morre of the Department of Botany and Plant Pathology, School of Agriculture.

The paper is planned for presentation at the Annual Meeting of the Indiana Academy of Science on November 6 at Terre Haute, Indiana. It is also proposed for publication in the Proceedings of that meeting.

The paper is presented to the Board for information and for approval of such presentation and publication. It will also be forwarded to the ISHC and the FHWA for review, comment and approval of publication.

Respectfully submitted,

Harold L. Michael
Associate Director

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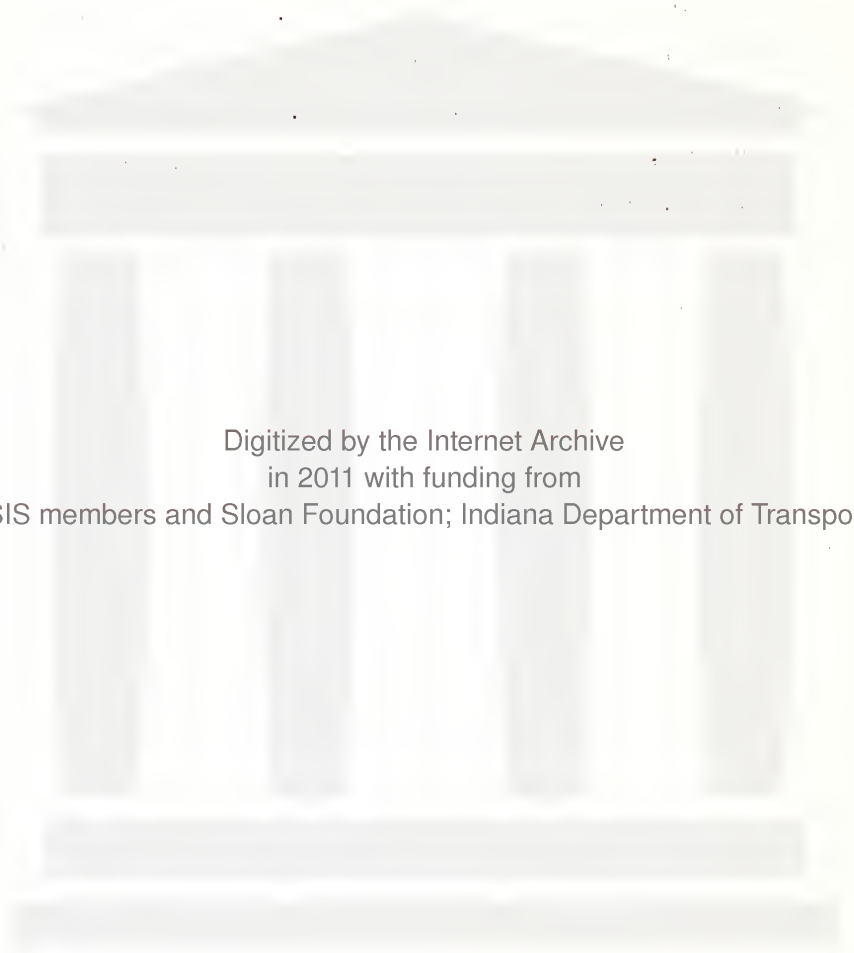
ABSTRACT

Two common herbicides, picloram or Tordon (4-amino-3,5,6-trichloropicolinic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid) and their salts exhibit low toxicity to fish. Certain formulated derivatives (especially esters) tended to be more toxic than acid salts as was an impurity from technical picloram. Even with picloram containing impurities, adaptive and/or detoxification responses by the fish were indicated. These herbicides (picloram and 2,4-D) seem to present a low potential hazard to fish from normal agricultural or industrial practice.



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Growth regulating herbicides have been developed over the past 30 years which control broad spectra of plant species. They are used extensively for control of brush and herbaceous species in non-crop areas as well as for weed control in agricultural and recreational areas and in yards and gardens. In addition to the standard herbicides used for these purposes such as 2,4-D and 2,4,5-T, a never material called picloram or Tordon herbicide has also received extensive testing and wide spread use in noncrop areas (Table 1). The mode of action of picloram is similar to that of 2,4-D and 2,4,5-T (3, 4, 5, 7, 13, 17, 19) but with a greater herbicidal effectiveness due to increased mobility and resistance to breakdown with the plant (5). As a consequence of its resistance to breakdown, picloram introduced into the biosphere may persist for a year or more (6, 8, 11, 16) in contrast to 2,4-D and 2,4,5-T which are broken down more rapidly (10). The present communication constitutes preliminary studies from our laboratory dealing with the toxicity of 2,4-D- and picloram- containing herbicides to green sunfish (Lepomis cyanellus) and is part of a more extensive program concerning the mode of action of herbicides of the auxin type, their derivatives and formulations, and their effects on aquatic ecosystems.

MATERIALS AND METHODS

Green sunfish (Lepomis cyanellus), 50-150 g, were maintained in pond water at 24° with continuous aeration and on artificial diets. Fish mortality was recorded and at the end of the study, fish were examined for tissue abnormalities. The livers were excised, weighed and sampled

for histological examination.

For electron microscopy, portions of the livers were fixed at 0-4° C in 2.5% glutaraldehyde (Fisher, Biological Grade) in 0.1 M sodium phosphate at pH 7.2 followed by a buffer rinse and post fixation in 1% osmium tetroxide in the same buffer. Specimens were dehydrated in a graded acetone series and embedded in Epon (22). Thin sections were post-stained with lead citrate (20) after being mounted on carbon-coated parlodion-covered grids and were viewed with a Philips EM-300. Magnifications are approximate.

RESULTS

With 2,4-D, neither the acid nor commercial salt formulations were toxic to green sunfish at a concentration of 5×10^{-4}M (110 ppm acid equivalent) (Table 2). However, at this same concentration, the butoxy ethanol ester of 2,4-D proved toxic after 60 minutes of exposure. Similar results were reported previously by Butler (Table 3) in tests using three salt water species of fish. With picloram, the 99% analytical grade material was also nontoxic at 5×10^{-4}M (1.2 ppm acid equivalent). However, both the 91% technical picloram and the 22% commercial formulation were toxic (Table 2) suggesting the presence of an impurity in these preparations. Similar toxicities of picloram to fish were reported by Kenaga for rainbow trout and other species (15) with the isooctyl ester being at least 10 times more toxic than the corresponding amine salts (Table 4).

In our studies, fish were quickly immobilized by 5×10^{-4}M technical picloram but the fish did not die. Within the first hour of picloram

treatment, the fish recovered motility and swam normally upon return to pond water without herbicide. If the fish were placed in technical picloram solutions for varying lengths of time and then returned to pond water, they recovered and began to swim normally. The recovery time varied as an approximately linear function of the treatment time (Figure 1). If after recovery, the fish were then given a second exposure to technical picloram, they would again recover but the recovery times were generally shorter (Figure 1). This trend continued through a 3rd exposure to technical picloram and after a 4th exposure many of the fish failed even to respond, i.e. 0 recovery time.

Liver changes were noted, even in fish maintained on a dose of technical picloram of 10^{-4} M (Table 4) which did not affect the swimming response. Ultrastructural changes associated with technical picloram toxicity or detoxification included a marked and conspicuous proliferation of the smooth endoplasmic reticulum (compare Figures 2 and 3). With longer times of treatment, liver degeneration was noted in some but not all of the treated fish with the ultrastructural pattern illustrated in Figure 4.

As a first step in determining the nature of the toxic impurity present in technical picloram, the following compounds were obtained through the courtesy of the Dow Chemical Company, Midland, Michigan:

1. 4-amino-3,5,6-trichloropicolinonitrile
2. 2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine
3. 4-amino-2,3,5,6-tetrachloropyridine
4. 6-amino-3,4,5-trichloropicolinic acid

These compounds are known to be present in technical picloram as impurities and compound 2 illustrated below, was found to be toxic (Figure 5) to fish.

2-(3,4,5,6-tetrachloro-2-pyridyl) guanidine

The other 3 impurities tested were not toxic to fish at a concentration of 10^{-4} M (Figure 5) and the swimming response of the fish in the presence of the compounds was normal.

DISCUSSION

With its relatively low toxicity of 8,200 mg/kg and its rapid excretial rate from the animal body, picloram or Tordon Herbicide seems to present no serious hazard to man or terrestrial animals (15). 2,4-D is even less toxic. The toxicities of these herbicides to birds has been reported to be low (15) and no effect on reproduction of either birds or fish have been noted (15). As summarized in this report, the acute toxicity of picloram or 2,4-D to fish is also low. However, the use of ester formulations tends to increase toxicity emphasizing the need for testing specific commercial formulations, combinations of herbicides and combinations of herbicides with other types of water pollutants.

Pure picloram seems to be virtually without effect on fish. The fact that commercial picloram or Tordon Herbicide has an LD_{50} to fish

in the range 10-500 ppm (depending on species, size and conditions of exposure) seems to arise from a toxic impurity present in technical and commercial picloram formulations. At concentrations at or near the LD_{50}, the loss of swimming response is reversible and upon repeated exposure the fish are able to adapt to the herbicide. The subacute response is accompanied by liver enlargement and formation of masses of smooth endoplasmic reticulum similar to those induced in response to a variety of drugs and pesticides (2, 13, 10). The proliferation of endoplasmic reticulum is associated with the induction of relatively non-specific steroid and drug hydroxylating enzymes. The action of these enzymes is to make the drugs more water soluble and more readily excreted from the organism. Again, the liver changes were studied only with the technical picloram and may very well be a response to one or more of the impurities present rather than to the actual herbicide.

It has been the practice of some investigators and many field biologists to regard all formulations of 2,4-D or picloram as equal in phytotoxicity and toxicity to fish. It is apparent from our data and the data of others (1, 15) that the formulation used is important. In shallow water, the amount of an ester formulation required to secure optimum results for weed control (Figure 6) may result in dramatic kill of fish, not from the basic herbicide, but the manner in which it is prepared for commercial use. However, as illustrated in Figure 6, the concentrations of salt formulations of even technical picloram which are toxic to fish approach the maximum solubility of picloram acid in water and are at least an order of magnitude greater (10-fold) than those which might be expected from accidental or direct contamination

of lakes or streams through normal use practices. On the same basis, the toxic concentrations reported in the study are 100-1000 fold higher than what might be expected in terms of water pollution resulting from runoff of treated soil or vegetation.

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TABLE 1. Characteristics of 2,4-D and picloram derivatives.

Compound	Abbreviation	Chemical Structure*
2,4-Dichloro-phenoxyacetic acid	2,4-D	
4-Amino-3,5,6-trichloropicolinic acid	Picloram (Tordon**)	

* Acid (R=H); salt (R=metal ion or organic amine); ester (R=long chain alcohol).

** Registered trademark of the Dow Chemical Company

TABLE 2. Effect of herbicide formulations on the swimming response of green sunfish (Lepomis cyanellus)

Herbicide	Conc.	Formulation	Acid Equivalent	Average Response Time
2,4-D	$5X10^{-4}$ M	Acid	99%	no effect in 41 hr
		Li^{+} Salt	95%	no effect in 41 hr
		Isopropyl-Diethylanolamine Salt	28%	no effect in 41 hr
		Butoxyethanol Ester	43%	60 min
Picloram	$5X10^{-4}$ M	Acid	99%	no effect in 41 hr
		Technical	91%	5 min
		K^{+} Salt	22%	5 min

TABLE 3. Comparative Toxicity of 2,4-D Formulations to Fish (*Leiostomus xanthurus*, *Fundulus similus* and *Mugil cephalus*).*

Formulation	LC_{50} - ppm ae 48 hr Exposure
Acid	no effect at 50 ppm
Dimethylamine salt	no effect at 15 ppm
2-Ethylhexyl ester	no effect at 10 ppm
Propyleneglycolbutylether ester	4.5
Butoxyethanol ester	5.0

* From Butler (1).

TABLE 4. Comparative Toxicity of Picloram Formulations to Rainbow Trout (*Salmo gairdnerii*)*

Formulation	Equivalent	(LC_{50} - ppm ae) Exposure Time 24 hr	96 hr
Triisopropanolamine salt	55.8%	279	209
Triethylamine salt	70.5%	43	29
Isooctyl ester	68.3%	10	3

* From E. E. Kenaga (15).

TABLE 5. Effect of Technical Picloram (91%) on Liver Weight of Green Sunfish (Lepomis cyanellus).

Treatment	Weight of Liver, % of Body Weight*	
	5 Days	9 Days
Control	0.65 ± 0.28	0.56 ± 0.16
Technical picloram (91% acid) 10^{-4} M	1.36 ± 0.61	1.08 ± 0.36

* Averages from 10 fish ± standard deviation.

LIST OF FIGURES

Figure 1. The relationship between exposure time to 5x10-4m Technical picloram (91% acid) and subsequent recovery of normal swimming response of green sunfish (Lepomis cyanellus).

Figure 2. Electron micrograph of fish (Lepomis cyanellus) hepatocytes. Of interest are endoplasmic reticulum profiles, most of them rough surfaced in stacked or whorled arrays interspersed with short tubular or vesicular profiles of smooth endoplasmic reticulum. Most if not all mitochondria of fish hepatocytes are surrounded by endoplasmic reticulum. Favorable planes of section (not illustrated) show continuity between the outer mitochondrial membrane and the enveloping cisterna of endoplasmic reticulum.

Figure 3. Fish hepatocytes after 24 hours exposure to 10^{-4}M (91% acid) technical picloram. Large expanses of a tubular network of smooth endoplasmic reticulum are evident. This type of smooth endoplasmic reticulum is never found in normal hepatocytes but is commonly induced by the administration of drugs (13).

Figure 4. Fish hepatocyte after 5 days of exposure to 10^{-4}M technical (91% acid) picloram. Most of the endoplasmic reticulum appears vesiculated and mitochondria appear swollen.

Figure 5. Green sunfish (Lepomis cyanellus) treated with various impurities from technical picloram (see text). Impurity 2 was toxic. In solutions containing the other three compounds, the fish maintained a normal swimming response.

Figure 6. Diagram illustrating some biological responses to picloram and 2,4-D herbicides over the range 0 to 3 X 10^{-3}M. The midportion of the diagram shows an expansion of the range 0 - 10^{-5}M and the bottom scale is an expansion of the range 0 to 10^{-6}M.